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First human exposure to exogenous single-dose oral estetrol in early postmenopausal women

M. Visser, C. F. Holinka and H. J. T. Coelingh Bennink*

Pantarhei Bioscience, Zeist, The Netherlands; *PharmConsult®, New York, NY, USA

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ABSTRACT

Objective To evaluate the safety, tolerability, pharmacokinetics and effect on gonadotropins of a single escalating dose of estetrol (E_4).

Methods A first-in-human study with E_4 was performed in healthy early postmenopausal women. Four single doses of 0.1, 1, 10 and 100 mg E_4 were evaluated in study groups of eight subjects each, of whom six received active treatment and two placebo treatment. Safety and tolerability were documented and several pharmacokinetic parameters were determined, as were the plasma levels of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) (pharmacodynamics). The next higher-dose group was enrolled after pharmacokinetic evaluation and confirmed safety of the previous group.

Results After oral intake, the plasma concentrations of E_4 showed a steep increase, followed by a sharp decline and a secondary increase at all dose levels. Estetrol was distributed and reabsorbed during the first 18 h after oral intake. The terminal elimination phase started at 24 h post-dose and half-life ($t_{½}$) ranged in the 10 mg group between 19 and 40 h (mean 28.4 h, median 28.8 h) and in the 100 mg group between 18 and 60 h (mean 28.0 h, median 20 h), indicating a dose independency of the half-life.

The pharmacokinetic parameters also demonstrated a high dose–response relationship and showed excellent consistency and low variability within the dose groups.

The pharmacodynamic data showed a dose-dependent inhibition of plasma LH levels by E_4. A profound and sustained inhibition of FSH levels, lasting over 168 h, was observed in the 100 mg dose group (FSH was not measured in the other dose groups).

Estetrol was well tolerated at all dose levels and no safety problems were encountered.

Conclusions Estetrol is orally absorbed and bioavailable with a strong dose–response relationship suggesting high oral bioavailability. Interindividual variations of plasma levels are low. The elimination half-life of 28 h suggests slow metabolism of E_4. The pharmacodynamic pattern complies with enterohepatic recirculation. Estetrol has a profound central inhibitory and dose-dependent effect on gonadotropins, confirming its biological potency.

INTRODUCTION

Estetrol is a steroidal hormone, produced by the human fetal liver during pregnancy only. This natural hormone was discovered in urine of pregnant women by Egon Diczfalusy and

Correspondence: Professor H. J. T. Coelingh Bennink, Pantarhei Bioscience, PO Box 464, 3700 AL Zeist, The Netherlands

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co-workers at the Karolinska Institute in Stockholm in 1965[1]. Structurally, estetrol is an estrogenic steroid with four hydroxyl groups, explaining the acronym E_4. Estetrol has weak estrogenic effects in a number of experimental systems. Competitive binding studies revealed relatively low affinity to nuclear and cytosolic estrogen receptors[2–4]. In the rodent uterus, E_4 exerted weak agonistic effects on weight, alkaline phosphatase activity and the induction of the progesterone receptor[5,6]. It promoted growth and progesterone receptor induction in cultured estrogen-responsive human breast cancer cells (MCF-7), but over 50 times higher concentrations were required for E_4 to elicit effects comparable to those of estradiol (E_2)[7]. In pregnant women, E_4 has been isolated in maternal urine as early as week 9 of gestation[8,9]. During pregnancy, E_4 has been detected at steadily increasing concentrations in maternal plasma, reaching nanomolar concentrations in the maternal circulation at parturition and 10–20 times higher levels in the fetal circulation[10,11].

More details concerning the history of E_4 and data from studies in the period from 1965 to 1984 have been summarized in a review paper[12].

In recent years, E_4 has been studied in predictive, validated, pharmacological *in vivo* rat models. High oral absorption and bioavailability with a 2–3-h elimination half-life have been established[13]. Dose-dependent pharmacodynamic effects were observed on vagina[14], uterus[13], bone mass and strength[13], hot flushes[15], and ovulation inhibition[16], with a potency 10–20 times lower than ethinylestradiol. However, in breast cell and tumor models, E_4 appeared to act as an estrogen antagonist (in the presence of estradiol), with a potency comparable to tamoxifen and ovariectomy[17]. Based on this profile and its origin, E_4 is classified as a human fetal SERM (selective estrogen receptor modulator).

The present first-in-human study was performed to investigate the safety, tolerability, pharmacokinetic and pharmacodynamic effects on gonadotropins of a single rising dose of E_4 in healthy postmenopausal women.

METHODS

Study design and subjects

The study was conducted at the Clinical Pharmacology Unit of Kendle International Inc., in Utrecht, The Netherlands, and complied with the last revision of the Declaration of Helsinki and the ICH guideline for Good Clinical Practice. The study was approved by an independent Ethics Committee and all subjects gave written informed consent.

The study was a double-blind, randomized, placebo-controlled, first-in-human phase IA study. The objective of the study was to evaluate the safety, tolerability, pharmacokinetics, and effect on the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) of a single escalating dose of E_4.

In this study, 32 healthy postmenopausal female volunteers between 53 and 69 years of age were enrolled. Menopause was defined as $\geq$12 months amenorrhea or 6 months amenorrhea with serum FSH levels $\geq$40 IU/l and serum E_2 $<$73 pmol/l. The treatment groups were comparable with respect to the demographic parameters and characteristics.

In each dose group, six subjects received active treatment and two subjects received placebo. After screening, all subjects of the first and lowest dose group were admitted to the Clinical Unit for approximately 60 h of intensive safety monitoring and frequent sampling for pharmacokinetics and gonadotropin estimations. Safety and tolerability were documented by monitoring (serious) adverse events (AE), physical examinations including vital signs, and safety laboratory assessments (hematology, biochemistry, urinalysis). Volunteers were to return to the clinic for a visit 72 h after dosing. Follow-up was scheduled 2 weeks $\pm$ 2 days after dosing.

Medication

Estetrol (estra-1,3,5(10)-triene-3,15α,16α,17β-tetrol; molecular formula $C_{18}H_{24}O_4$, relative molecular mass 304.38) was synthesized by Chemshop (Weert, The Netherlands). Estetrol was 99.2% pure and did not contain any detectable E_2, as determined by HPLC analysis (results not shown). Active and placebo formulations were manufactured by ACE Pharmaceuticals BV (Zeewolde, The Netherlands). The study medication was formulated as a solution (propylene glycol/water mixture) and was delivered in separate bottles for each volunteer.

Dosing

Four groups of eight subjects each were treated with 0.1 mg, 1 mg, 10 mg, and 100 mg E_4, respectively. In each group of eight subjects, six subjects were randomly and double-blind assigned

to active treatment with E_4 and two subjects to treatment with placebo, i.e. a total of 24 subjects received E_4 and eight subjects placebo.

The next higher dose group was enrolled after pharmacokinetic evaluation and confirmed safety of the previous group and approval by an independent Ethical Committee.

Assessments

Safety was monitored on a continuous basis during admission to the Clinical Unit. A physical examination was scheduled at 48 h after dosing, if indicated, and at follow-up. Vital signs and ECG were performed just prior to dosing, 2 h after dosing and every 24 h, with a last evaluation just prior to discharge. Adverse events were monitored and documented continuously.

Frequent blood sampling for pharmacokinetics in the first three groups was performed using the following schedule: 0 (just prior to dosing), 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, 48, and 72 h after dosing. Because of the long elimination half-life observed in the first three groups, the sampling schedule for the 100 mg dose group was adjusted to: 0 (just prior to dosing), 0.25, 0.50, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, and 168 h after dosing, i.e. up to 7 days after dosing. Plasma samples for evaluation of E_4 levels were stored at $-20°C$ and shipped for analysis as one batch.

Additional blood samples were taken for evaluation of LH in groups 1–3 following the same schedule as the pharmacokinetic sampling. Because of the dose-dependent inhibitory effect on LH levels observed in the first three groups, the protocol was amended to also include FSH estimations in the 100 mg group. All samples were stored at $-20°C$ until analysis and shipped for analysis as one batch.

Assays

Estetrol was analyzed under Good Laboratory Practice (GLP) conditions by Xendo Laboratories, Groningen, The Netherlands, using a methodology especially developed for this study. Plasma (1 ml) was extracted with 2 ml of extraction solvent (hexane : ethylacetate, 50 : 50) after addition of internal standard and buffer. The extract was evaporated and redissolved in mobile phase and subsequently analyzed on an LC-MS/MS system (Sciex AP14000). The mobile phase was a gradient of ammonium formate buffer (10 M; pH = 5) and methanol. Quantification was performed on the basis of validated calibration curves. The technical quality of the obtained results was based upon the results of concomitantly analyzed quality control samples. The lower limit of quantification (LOQ) with this method was 25 pg/ml.

LH and FSH levels were analyzed at the Central Laboratory of the Canisius-Wilhelmina Ziekenhuis, Nijmegen, The Netherlands by the electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany).

Pharmacokinetic evaluation

Data of all subjects were included for the pharmacokinetic analysis. Two protocol deviations were noticed: two samples were centrifuged 1 and 2 h later than planned, respectively. The values of those two samples did not affect the respective concentration profiles and were included in the analysis. Six samples deviated more than 5% from the target time. In these cases, actual times were used.

The plasma concentration of E_4 was below the LOQ in all except three samples in the lowest dose group (0.1 mg) and the E_4 data of this group are not presented. Two E_4 plasma concentration values in the other groups were not used for AUC calculations and descriptive statistics, as they were not consistent with the remainder of the data for those individuals or the data of the group as a whole. Reliable estimates for the terminal elimination half-life values in the 1 mg group were obtained for two subjects only because the correlation coefficients to determine the elimination rate constant were below the prespecified limit of 0.9 in the other subjects. A reliable estimate of $AUC_{0-\infty}$ was obtained for one subject only, because the percentage AUC extrapolation was above the prespecified limit for the remainder of the 1 mg group.

Plasma pharmacokinetic parameters were calculated in WinNonlin, edition 3.3, using a non-compartmental model and according to a preset statistical plan. The following pharmacokinetic parameters were evaluated: C_{max} and t_{max} (peak concentration and time of occurrence directly taken from the measured plasma E_4 concentrations); $t_{½}$ (terminal elimination half-life estimated by fitting a straight line through data points, at least three data points and a correlation coefficient ≥ 0.9, in the terminal elimination phase); $AUC_{0-tlast}$ and $AUC_{0-\infty}$ (area under the concentration–time curve from time zero to the point with the last measurable concentration,

using the linear trapezoidal rule and area under the concentration–time curve from time zero to infinity calculated as $AUC_{0-\infty} = AUC_{0-t\text{last}} + AUC_{t\text{last}-\infty}$; $AUC_{t\text{last}-\infty} = C_{t\text{last}}/\lambda_z$ where $C_{t\text{last}}$ was the measured concentration at time t_{last} and λ_z was the elimination rate constant during the terminal elimination phase); CL/*F* and V_z/F (fractional clearance, calculated as dose/$AUC_{0-\infty}$, and fractional distribution volume during the terminal elimination phase, calculated as $CL/F/\lambda_z$).

Pharmacodynamic evaluation

LH levels were listed per dose group, subject and treatment day for all dose groups. In addition, summary statistics were generated per dose group and treatment day.

For the 100 mg dose group, all LH and FSH pharmacodynamic parameters, as described in the pharmacodynamic analysis section, were listed per dose group, subject and treatment day. In addition, summary statistics were performed.

Safety evaluation

All safety data from the in-treatment period, defined as the time from the first administration of the trial medication until follow-up, were used for the safety analysis.

The (serious) AEs, the number of subjects who presented with each AE, the incidence of each AE during the in-treatment period and the incidence of the same events occurring during the period between screening and start of treatment were recorded.

Statistics

Descriptive statistics were used for the demographic data as well as the plasma E_4 concentrations by time point, provided that at least two-thirds of the values were greater than or equal to the LOQ. For calculation and plotting of the mean concentration–time curve, concentrations lower than LOQ were replaced by 0.5*LOQ. An exploratory analysis of dose proportionality was performed by plotting $AUC_{0-\infty}$ versus dose.

RESULTS

Subject characteristics

No differences in demographic data were observed between total groups or between groups of actively treated subjects and the group of subjects receiving placebo. In summary, 32 healthy postmenopausal volunteers participated in the study with a mean age of 59.9 years (range 53–69 years), mean weight of 73.2 kg (range 57.4–95.2 kg) and mean body mass index of 25.8 kg/m^2 (range 21.9–30.8 kg/m^2).

Safety and tolerability

No clinically relevant side-effects occurred. The reported side-effects were mild in all but one subject, who reported moderately severe back pain, considered non-related to study drug treatment. The incidence and nature of side-effects reported were comparable in all groups including the group of subjects receiving placebo. One subject in the 100 mg group on active treatment developed a mild urticarious rash on face and chest approximately 15 min after dosing, which was considered an allergic reaction. The subject recovered quickly and uneventfully after antihistaminic treatment. Skin tests and intracutaneous injections with a series of dilutions of study drug, with and without active ingredient, utilizing the original batch did not provoke an allergic reaction to the compound or the vehicle used.

No clinically relevant changes from baseline were observed in vital signs, ECG, clinical laboratory evaluation including extensive blood biochemistry, hematology, and urinalysis.

Pharmacokinetics

The mean plasma E_4 concentrations versus time plots of the 1 mg, 10 mg, and 100 mg group are presented in Figure 1. The plasma concentration profiles showed a very steep increase and a subsequent sharp decline with all dosages administered. No lag time was observed in any subject. Estetrol was distributed quickly and reabsorbed during the first 18 h after oral intake. One secondary peak was noticed in all and two secondary peaks in occasional subjects. The typical profile of distribution and reabsorption was evident at all dose levels investigated. A slow phase of final elimination started approximately 24 h after dosing. The individual concentration profiles showed little interindividual variation and were highly consistent within each dose group.

A summary of pharmacokinetic parameters is presented in Table 1. Maximum plasma concentrations of E_4 after oral intake were observed at 15 min in the 1 mg group, between 15 and 30 min in the 10 mg group and between 15 and 60 min in the 100 mg group. The maximum concentration

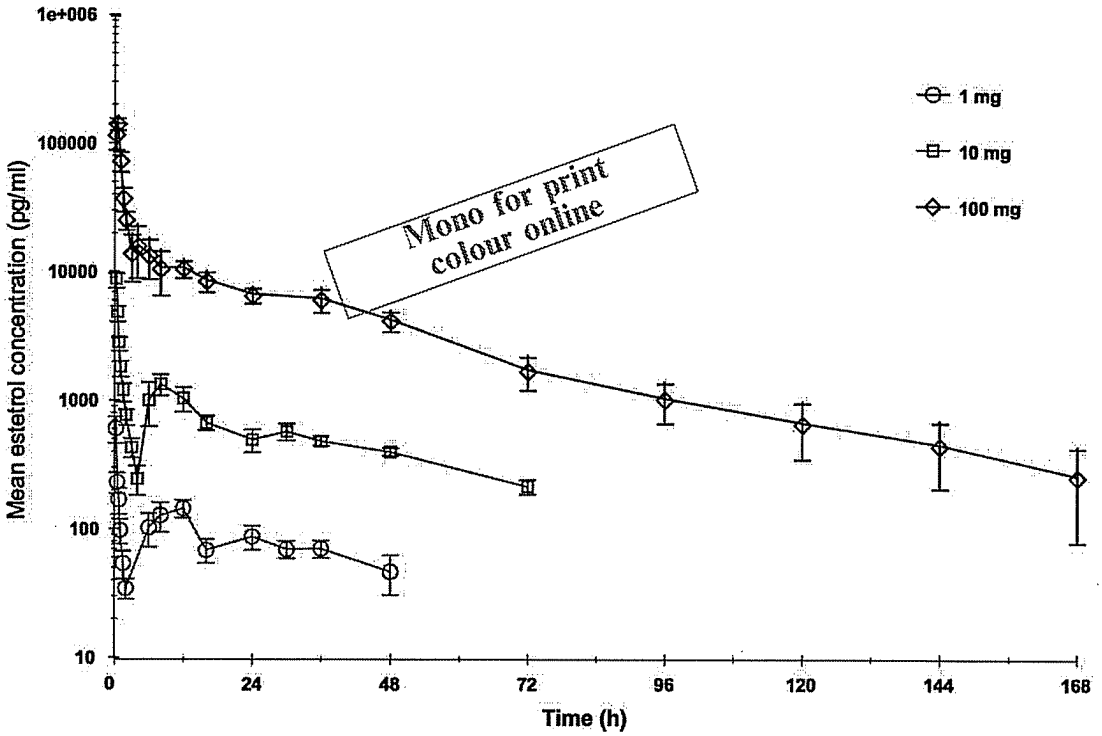

Figure 1 Mean estetrol plasma levels (± standard deviation) of the 1, 10 and 100 mg dose groups

of E_4 ranged between 0.39 and 1.2 ng/ml in the 1 mg group, between 5.4 and 11.6 ng/ml in the 10 mg group, and between 119 and 213 ng/ml in the 100 mg group. The $AUC_{0\text{-}tlast}$ ranged between 2.6 and 8.4 h*ng/ml in the 1 mg group, between 26.8 and 54.8 h*ng/ml in the 10 mg group, and between 460 and 927 h*ng/ml in the 100 mg group. Median $AUC_{0\text{-}tlast}$ values were 3.0, 43.5 and 640 h*ng/ml in the three groups, respectively.

The terminal elimination phase appeared to set in at 24 h after dosing. The terminal half-life ranged between 19 and 40 h in the 10 mg group and between 18 and 60 h in the 100 mg group, with medians of 29 and 20 h in the two groups, respectively. The values computed from the two evaluable subjects in the 1 mg group (31 and 32 h) were consistent with the above results.

The $AUC_{0\text{-}\infty}$ calculated from the data of three subjects in the 10 mg group ranged between 44.7 and 64.9 h*ng/ml, median 50.2 h*ng/ml, and between 462 and 1024 h*ng/ml in the 100 mg group, median 646 h*ng/ml, which is dose proportional.

The geometric means of the fractional distribution volume and fractional clearance were similar in the 10 mg and 100 mg groups, confirming dose-independent pharmacokinetics. The clearance data (largest range 98–224 l/h) suggest that other processes than hepatic clearance are involved, as the clearance exceeds liver blood flow.

Gonadotropins

Serum LH levels were measured in all dosing groups following the pharmacokinetic sampling schedule. No effect on LH levels was observed after dosing with 0.1 and 1.0 mg E_4. LH was slightly decreased after 10 mg in all subjects as compared to placebo. Levels were back to baseline after approximately 24 h. In the 100 mg group, an immediate decrease (lowest point 4–8 h post-dose, mean decrease of 18 IU/l) was observed in all subjects as compared to placebo and returned to baseline at approximately 72 h post-dose. The maximum mean suppression was 48% at 4 h after dosing. A second LH trough of 37% is seen at 12 h after dosing, synchronous with the mean E_4 levels. A slight potential rebound is observed between 48 and 72 h after dosing, until a maximum of 18% at 168 h after dosing. The

Table 1 Summary of pharmacokinetic parameters of estetrol (E_4)

	t_{max} (h)	C_{max} (ng/ml)	$AUC_{0\text{-}tlast}$ (h*ng/ml)	$t_{½}$ (h)	$AUC_{0\text{-}\infty}$ (h*ng/ml)	V_Z/F (l)	CL/F (l/h)
1 mg E_4							
n	6	6	6	0	0	0	0
Mean	0.253	0.5897	4.081	–	–	–	–
SD	0.0082	0.28372	2.2510	–	–	–	–
Minimum	0.25	0.387	2.63	–	–	–	–
Median	0.25	0.519	3.04	–	–	–	–
Maximum	0.27	1.12	8.42	–	–	–	–
G mean	–	0.5485	3.702	–	–	–	–
CV% G mean	–	40.2	47.6	–	–	–	–
10 mg E_4							
n	6	6	6	6	3	3	3
Mean	0.333	8.928	42.27	28.42	53.25	6191.8	192.4
SD	0.1291	2.731	10.170	7.587	10.478	935.37	35.43
Minimum	0.25	5.42	26.8	18.9	44.7	5438	154
Median	0.25	9.39	43.5	28.8	50.1	5899	199
Maximum	0.50	11.6	54.8	40.4	64.9	7239	224
G mean	–	8.554	41.16	–	52.59	6146.4	190.1
CV% G mean	–	33.7	26.4	–	19.3	14.8	19.3
100 mg E_4							
n	6	6	6	6	6	6	6
Mean	0.542	162.2	661.9	28.02	681.3	5792.6	161.4
SD	0.2458	39.99	205.18	16.350	232.71	1603.62	51.99
Minimum	0.25	119	460	17.5	462	3434	97.6
Median	0.50	148	640	20.1	646	5689	163
Maximum	1.00	213	927	60.0	1024	8453	216
G mean	–	158.3	635.5	–	649.2	5602.4	154.0
CV% G mean	–	24.3	32.2	–	34.9	29.5	34.9

SD, standard deviation; G mean, geometric mean; CV, coefficient of variation

actual levels and percentage change of the LH levels of the 100 mg group are shown in Figure 2.

Serum FSH levels were measured in the 100 mg group only and the actual levels and percentage change of the FSH levels of this dose group are presented in Figure 3. A profound and sustained decrease of FSH was observed in all subjects, with the lowest point at 48 h post-dose as compared to placebo. The levels were back to baseline at approximately 168 h (1 week) post-dose. The maximum suppression of the FSH levels was 41% at 48 h after dosing.

In conclusion, suppression of LH is closely and inversely related to the plasma E_4 levels, whereas suppression of FSH is more sustained.

DISCUSSION

The results from this first-in-human study with E_4 of an escalating single oral dose of this steroid demonstrate that, in the dose range tested, it is safe and well tolerated, has a high and dose-proportional oral bioavailability with a consistent plasma profile due to little interindividual variation, and a remarkably long terminal elimination half-life of about 28 h; a single dose of E_4 suppresses serum gonadotropins dose-dependently (LH) and for long periods of time up to 1 week (FSH).

No safety concerns have arisen during this study. This finding is in line with what could be expected based on the nature of the compound. Estetrol is produced in nature in large quantities by the human fetal liver, which expresses 15α-hydroxylase activity, necessary to attach a fourth hydroxyl group to estriol (E_3). Soon after birth, the capacity of the liver to express this enzyme is turned off. Therefore, long-term human exposure is natural during vulnerable episodes in life, i.e. fetal development in both genders and pregnancy in adult females. Fetal and maternal blood levels are increasing during pregnancy and peak at term

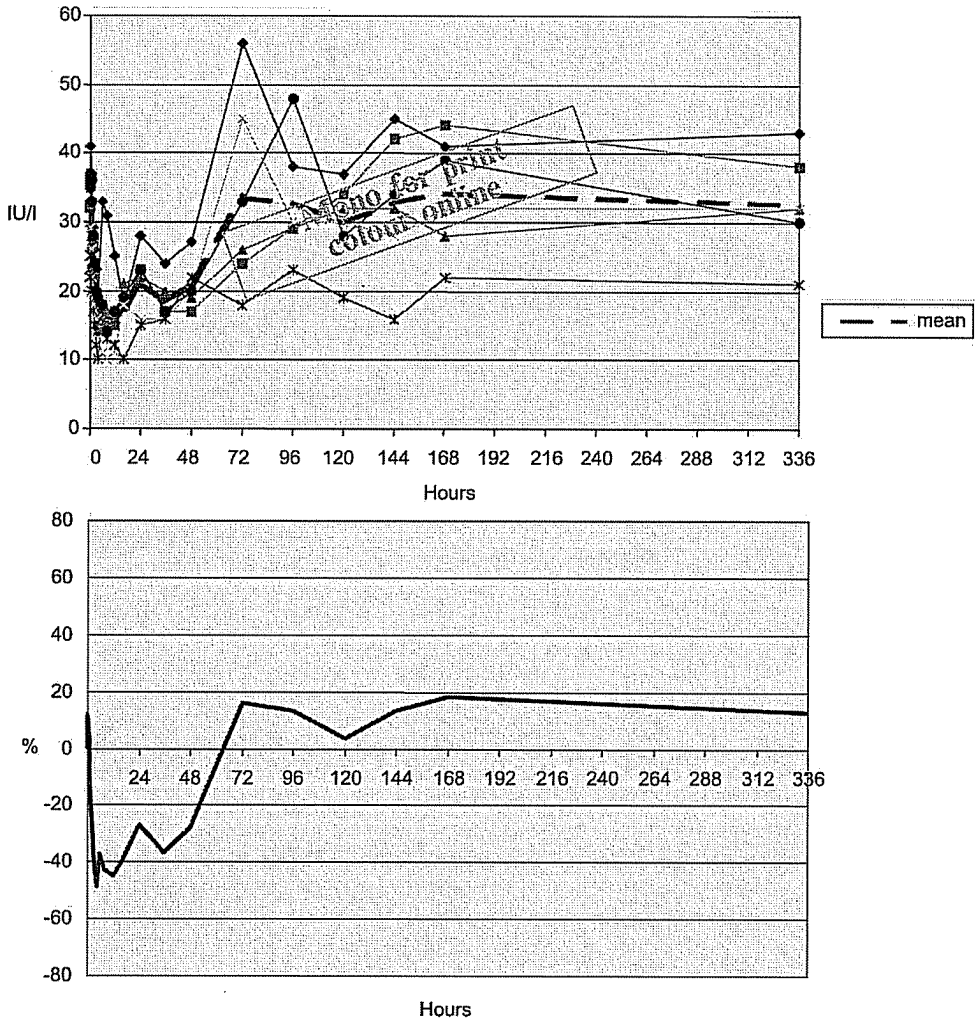

Figure 2 The actual individual and mean (upper figure) LH levels and the percentage change of the mean (lower figure) LH levels in the 100 mg dose group

with plasma estetrol levels in the fetus averaging an AUC_{0-24} of 550 h*ng/ml and maternal levels around 10–20 times lower than in the fetus[10,11].

After oral administration, E_4 is absorbed rapidly with peak levels 15–60 min after dosing. Thereafter, E_4 levels decline rapidly, followed by a second peak indicating fast distribution and reabsorption. Then, a slow elimination phase starts approximately 24 h after intake. This profile of plasma E_4 levels was observed consistently in all subjects studied and is rather typical for enterohepatic recirculation, known to occur with estrogenic steroids. However, temporary storage in lipid tissue cannot be ruled out completely yet.

Given the concurrence of plasma peak values and the timing of the first sampling point at 15 min after dosing, all subjects in the 1 mg dose group and several subjects in the 10 and 100 mg dose groups could actually have had their peak E_4 concentration even at an earlier point in time, not captured by the sampling schedule employed. This would lead to underestimation of the C_{max} and the AUC calculation. It should also be noticed that the final samples taken from subjects in the 10 mg group at 72 h after dosing were between 127 and 264 pg/ml, which is well above the LOQ of 25 pg/ml. The t_{last} in this group, therefore, is not the last sample with a concentration just above the LOQ, also leading to an underestimation of the factual $AUC_{0\text{-}tlast}$. This did not occur in the 100 mg group since sampling was extended in this group, based on the findings with 10 mg E_4. Taking into

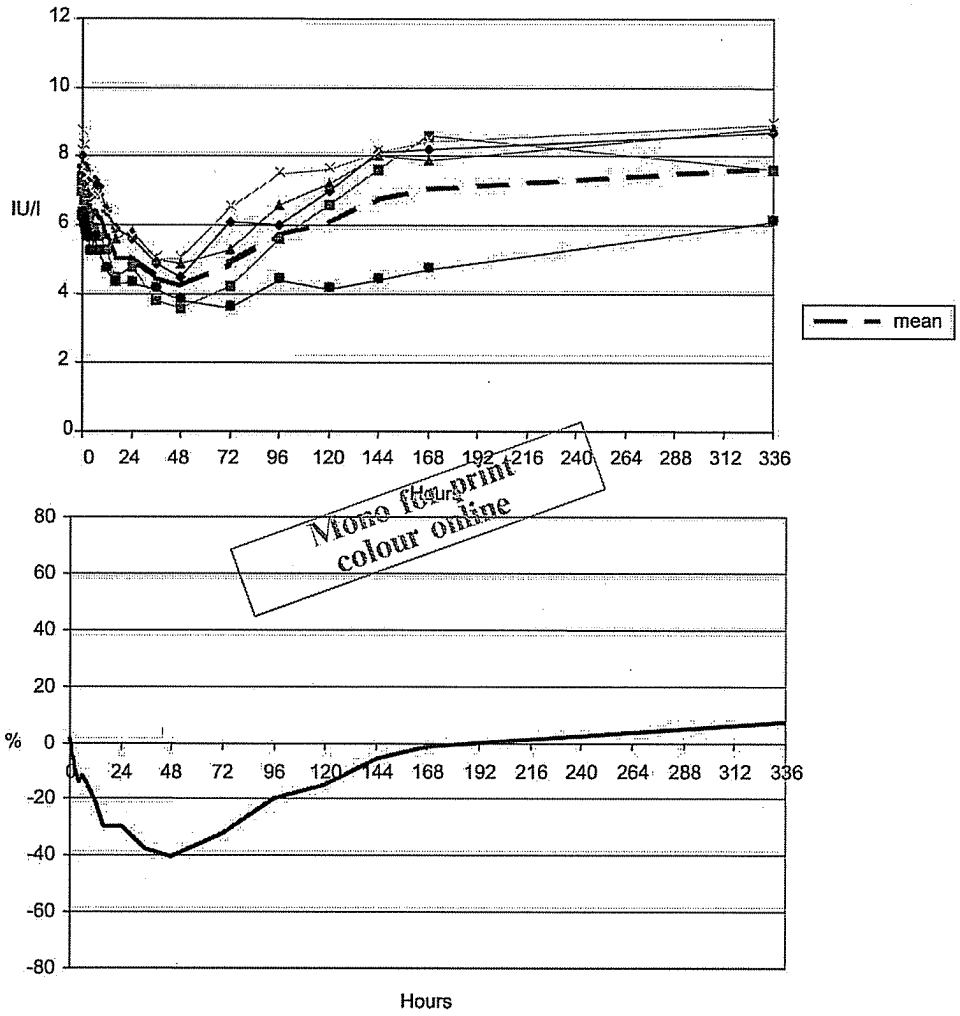

Figure 3 The actual individual and mean (upper figure) FSH levels and the percentage change of the mean (lower figure) FSH levels in the 100 mg dose group

consideration these remarks, it is concluded that E_4 shows dose linearity after oral intake in the dose range investigated for all criteria studied. The high and fast dose-dependent initial peak level of E_4 after oral intake may be useful for applications in which an immediate clinical effect is the aim.

Focusing on oral administration, the pharmacokinetic and metabolic characteristics of E_4 are very different from those of estrogens currently used, both natural estrogens such as human E_2 and E_3 and conjugated equine estrogens from pregnant mares, as well as synthetic estrogens such as ethinylestradiol. Several review papers on the pharmacokinetics of estrogens are available[18–20]. An important difference between the other human estrogens and E_4 is the terminal elimination half-life. Whereas E_4 is shown in this study to have a half-life of about 28 h, the half-lives of E_2 and E_3 are 1–2 h and 20 min, respectively. Therefore, the most widely used E_2 has to be micronized or esterified to extend its half-life and make it suitable for oral use in the human, whereas E_3 can be used for local (vaginal) administration only. The terminal half-life of ethinylestradiol is about 20 h[19,21]. Therefore, ethinylestradiol does not require modification and is suitable for use as a once-a-day drug in the human; the same is expected for E_4.

The pharmacokinetic profile of E_2 is extremely complex due to its high first-pass metabolism, its extensive enterohepatic recirculation through

biliary excretion of conjugated estrogens, followed by intestinal reabsorption, and its partial and reversible conversion to other compounds (estrone, estrone sulfate), that may be pharmacologically active[22]. This process is further complicated by binding of estrogens to blood carrier proteins, such as albumin and especially to sex hormone binding globulin (SHBG)[23]. Estradiol is partially inactivated by binding to SHBG (38%) and loosely bound to albumin (60%), resulting in a biologically free fraction of about 2%[24]. Moreover, the levels of SHBG may be increased by the administered estrogen itself, leading to very complex metabolic and pharmacological dynamics. The pharmacology of conjugated equine estrogens is even more complex and to a large extent not fully elucidated. Equine estrogens are a cocktail of many different pharmacologically active estrogenic compounds, all of them also undergoing extensive metabolic conversions comparable to those of E_2[25]. Ethinylestradiol has a bioavailability of 38–48% due to a marked metabolism in the gut mucosa and during first liver passage, whereas ethinylestradiol also undergoes extensive enterohepatic recirculation[21]. High local concentrations during the first liver passage of ethinylestradiol exist, due to the relatively slow transformation into inactive metabolites in hepatocytes and result in pronounced effects on hepatic metabolism (e.g. on lipoproteins, clotting factors, SHBG). Ethinylestradiol is bound to albumin and hardly to SHBG, indicating that circulating ethinylestradiol is rather easily bioavailable. Oral administration of all these estrogens is associated with large inter- and intraindividual variations of plasma levels, which complicates appropriate individual dosing.

As mentioned, the results of the current study show that the pharmacokinetics of E_4 suggest enterohepatic recirculation comparable to other estrogens. Differently from other natural estrogens, however, E_4 is partially excreted via the kidneys[26,27]. Also, other metabolic effects of E_4 seem to differ considerably. First of all, E_4 is an end-stage product of metabolism and is not converted into other active metabolites, including estrogens such as estrone (E_1), E_2 or E_3. Second, *in vitro* E_4 does not bind to SHBG[28] and only moderately to albumin. This means that, after oral dosing, the circulating E_4 is readily bioavailable. In this respect, E_4 is comparable to ethinylestradiol. Combined with the low inter- and intraindividual variation of the plasma levels of E_4 and with the dose linearity, both shown in this study, it may become possible to relate plasma concentrations of E_4 with clinical effects and achieve individual dose adjustment based on plasma levels, which is impossible with all presently available estrogens including ethinylestradiol.

In this study in early postmenopausal women with high gonadotropin levels, LH was measured to obtain a first impression whether oral E_4 has any dynamic effects. LH serum levels are known to be very sensitive to suppression by steroids, but frequent measurements are needed since LH has a short half-life of about 20 min. Therefore, LH was measured following the kinetic sampling schedule. When, in the first three dose groups, a dose-dependent decrease of LH was found, it was decided to measure FSH in the last 100 mg dose group too. This high single dose of E_4 induced a prompt and profound suppression of LH levels in all subjects treated (Figure 2) and a more gradual and sustained suppression of FSH levels, returning to baseline after 7 days, also observed in all women treated (Figure 3). This difference in effect is most likely due to the much longer half-life of FSH (28 h) as compared to LH (20 min). These data confirm that E_4 has biological effects when administered orally in the human. More specifically, the suppressive effect on LH and FSH supports the potential use of E_4 for oral contraceptive purposes.

The effect of E_4 on gonadotropins in the human is rather strong and contradicts the conclusion in the past that E_4 is a weak estrogen. This conclusion was based on low receptor affinity[2–4] and on the results of *in vitro* and brief parenteral *in vivo* experiments in the period 1965–1984[12]. The pharmacokinetic properties of E_4 reported in this study, especially its long elimination half-life, offer an explanation for the unexpectedly high biological potency of E_4.

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Conflict of interest M.V. is a shareholder of Pantarhei Bioscience; C.F.H. has financial interests in estetrol; H.C.B. is CEO and shareholder of Pantarhei Bioscience.

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